Variation in Root Development Response to Flooding among 92 Soybean Lines during Early Growth Stages

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Abstract: Maintaining root function is crucial for favorable plant growth under flooding. The genetic variation in the response of root development to flooding is unclear, because measurement of root growth is time consuming, especially with numerous lines. To overcome the methodological problems and to reveal the effect of flooding on root development and its genetic variation, we developed a new capillary watering system without soil medium and raised cotyledon-stage seedlings of 92 soybean lines with and without flooding. After 7 days of flooding, dry weights (DW) and root characteristics were determined and the results were compared with those in non-flooded plants. The root DW decreased linearly with decreasing total root length and root surface area, and the degree of damage varied greatly among lines. Short-term flooding inhibited root elongation and branching, but not in flood-tolerant lines.

Key words: Early growth stage, Flood-tolerance, Genetic variation, Root development, Soybean (Glycine max).

Both natural and anthropogenic flooding cause yield losses in agriculture (Rosenzweig et al., 2002). Like most crops, soybean (Glycine max (L.) Merr.), which is the most widely grown legume in the world, is susceptible to flooding, which reduces plant biomass and yield (Sugimoto et al., 1988; Scott et al., 1989; Linkemer et al., 1998; Bacanamwo and Purcell, 1999a; Henshaw et al., 2007a, b; Rhine et al., 2010). In Eastern Asia, soybean is often grown in upland fields converted from paddy fields (Lee et al., 2003; MAFF, 2004), and is generally sown in the spring to early summer monsoon season, placing the seedlings at risk of flood damage. Even short-term flooding can inhibit or kill seedlings, leading to serious yield losses.

Because roots acquire nutrients and water and synthesize organic acids, amino acids, and plant hormones (Yang et al., 2004), root development is closely related to aboveground development (Yang et al., 2012). Flooding damages both root and shoot growth (Sallam and Scott, 1987; Araki et al., 2012), affecting root growth first (Sauter, 2013); for example, in soybean, flooding reduced root dry weight (DW) before it reduced shoot DW (Shimamura et al., 2003a). Therefore, maintaining root development is crucial for favorable plant growth under flooding.

Internal oxygen transport from the air to the roots is important for root survival and function (Armstrong, 1979). Flooded plants often suffer hypoxia or anoxia stress, because oxygen moves much more slowly in water ($\times 10^{-4}$) than in the air (Armstrong, 1979; Armstrong and Drew, 2002), and dissolved oxygen is quickly depleted: the oxygen concentration in a nutrient solution decreased by about 80% within 24 hr after transfer of wheat seedlings into the solution (Wiengweera et al., 1997). Under prolonged flooding, normal root development is replaced by the formation of adventitious roots in field crops such as wheat (Mano and Omori, 2007) and tomato (McNamara and Mitchell, 1990). Flood-tolerant species often form adventitious roots from submerged stems (Colmer and Voesenek, 2009). Removal of adventitious roots from the wetland plants Cotula coronopifolia and Mesinecetes brownii under flooding reduced whole-plant growth, indicating that the adventitious roots are of some benefit to plant growth during flooding (Rich et al., 2012). Soybean plants can develop adventitious roots de novo during flooding, but it takes time for the roots to form and function effectively: roots grew only 1 cm after 4 to 5 d (Thomas et al., 2005). During the early growth stages, flood-tolerant rice
Table 1. Average dry weight (mg ± SD) in 92 soybean lines at the cotyledon stage.

<table>
<thead>
<tr>
<th>Lines</th>
<th>Root DW</th>
<th>Shoot DW</th>
<th>Whole-plant DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kilatsune</td>
<td>120.7 ± 21.30</td>
<td>128.1 ± 27.94</td>
<td>249.9 ± 48.35</td>
</tr>
<tr>
<td>Timachi</td>
<td>132.0 ± 39.34</td>
<td>157.7 ± 11.43</td>
<td>326.5 ± 20.74</td>
</tr>
<tr>
<td>MD40</td>
<td>185.7 ± 50.13</td>
<td>154.3 ± 36.90</td>
<td>305.8 ± 47.63</td>
</tr>
<tr>
<td>Mizusawa</td>
<td>114.7 ± 14.38</td>
<td>121.3 ± 12.09</td>
<td>241.4 ± 22.07</td>
</tr>
<tr>
<td>Tsuchiyu</td>
<td>89.8 ± 37.27</td>
<td>113.4 ± 1.49</td>
<td>202.2 ± 29.67</td>
</tr>
<tr>
<td>COL/Aomori (1983/21)</td>
<td>146.6 ± 27.55</td>
<td>124.8 ± 17.09</td>
<td>277.2 ± 32.72</td>
</tr>
<tr>
<td>Tama-urara</td>
<td>114.6 ± 34.54</td>
<td>125.3 ± 22.72</td>
<td>215.3 ± 86.15</td>
</tr>
<tr>
<td>TH112-1</td>
<td>122.9 ± 21.08</td>
<td>110.3 ± 9.96</td>
<td>205.6 ± 20.26</td>
</tr>
<tr>
<td>Toyoichihara</td>
<td>120.9 ± 21.48</td>
<td>125.1 ± 14.93</td>
<td>234.1 ± 27.33</td>
</tr>
<tr>
<td>Hashidaizu</td>
<td>112.7 ± 49.81</td>
<td>131.1 ± 30.58</td>
<td>217.7 ± 24.62</td>
</tr>
<tr>
<td>Mizumoto park No. 3</td>
<td>96.3 ± 37.34</td>
<td>94.1 ± 48.18</td>
<td>203.6 ± 25.67</td>
</tr>
<tr>
<td>ToSIE</td>
<td>116.7 ± 27.31</td>
<td>111.0 ± 15.35</td>
<td>228.8 ± 32.71</td>
</tr>
<tr>
<td>Kunita</td>
<td>146.6 ± 29.90</td>
<td>130.1 ± 39.99</td>
<td>282.7 ± 44.36</td>
</tr>
<tr>
<td>Sugasato</td>
<td>48.4 ± 3.73</td>
<td>53.5 ± 28.37</td>
<td>148.0 ± 46.12</td>
</tr>
<tr>
<td>Naito</td>
<td>75.3 ± 18.97</td>
<td>70.0 ± 29.58</td>
<td>148.0 ± 46.12</td>
</tr>
<tr>
<td>Kataoka</td>
<td>31.9 ± 10.61</td>
<td>33.9 ± 25.76</td>
<td>31.9 ± 10.61</td>
</tr>
<tr>
<td>Koganei</td>
<td>18.0 ± 11.19</td>
<td>22.8 ± 25.67</td>
<td>22.8 ± 25.67</td>
</tr>
<tr>
<td>Kasei</td>
<td>13.0 ± 10.61</td>
<td>16.3 ± 25.76</td>
<td>16.3 ± 25.76</td>
</tr>
<tr>
<td>Kunita</td>
<td>19.0 ± 10.61</td>
<td>22.8 ± 25.67</td>
<td>22.8 ± 25.67</td>
</tr>
<tr>
<td>Yamada</td>
<td>31.9 ± 10.61</td>
<td>33.9 ± 25.76</td>
<td>31.9 ± 10.61</td>
</tr>
<tr>
<td>Koganei</td>
<td>18.0 ± 11.19</td>
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<td>Kunita</td>
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<td>22.8 ± 25.67</td>
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</tr>
</tbody>
</table>

Note: ± SD ranked according to the rate of inhibition of root DW.
1. **Materials and Methods**

   **Plant materials**
   
   We tested 92 soybean lines: 87 *Glycine max* and 5 *Glycine soja* Sieb. et Zucc. (Table 1). All belong to the *Glycine* subgenus *Soja*. *Glycine soja* is a progenitor of *Glycine max* (Hymowitz and Newell, 1980), and is often found in marshy areas such as riverbanks and lakesides, in disturbed sites, and on mountain slopes (Jin et al., 2006). Seeds of each line except Mizumoto Park Nos. 1 – 4 were collected at the National Institute of Crop Science, Tsukuba, Japan (36° 11′ N, 140° 56′ E), in 2008; and seeds of Mizumoto Park Nos. 1 – 4 were collected from a natural population in Mizumoto Park, Tokyo, Japan (35° 79′ N, 139° 87′ E), in 2009. All seeds were stored at 4°C until our experiments.

2. **Capillary watering culture system**

   For each line, 20 seeds coated with a fungicide (benomyl, 0.5% of dry seed weight) were sown in plastic pots filled with wet vermiculite to encourage the development of straight radicles. Seeds of lines with a hard seed coat were scored with a razor blade to promote water absorption. At 2 or 3 d after sowing, seed coats were removed, and several seedlings with a radicle 4 to 5 cm long were transplanted into each of two plastic trays (flooded or unflooded) for culture.

   Each plastic tray measured 386 mm × 256 mm × 135 mm (Fig. 1). Doubled filter papers were folded in half (inside, No. 1; outside, No. 4A; Advantec), and seedling roots were sandwiched between a pair of folded filter papers. The filter papers were hung over horizontal strings to place their edges in the water. The flooded tray was filled with 0.1% agarose solution (Fig. 1A, C), and the unflooded tray held 2 to 3 cm of water (Fig. 1B, C). To support the roots and reduce evaporation, the filter papers were sandwiched between black plastic boards (Fig. 1C).

3. **Flooding treatment under hypoxia**

   The flooding treatment was started 8 d after sowing. Three to five uniform seedlings per tray were selected at the cotyledon stage in each of four lines per tray were selected and the rest were removed (Fig. 1C). For the flooding treatment under hypoxia, the tray was filled with 0.1% agarose solution to just under the cotyledons (Fig. 1A, C). The solution was bubbled with N₂ gas to reduce the dissolved oxygen concentration from about 3 mg L⁻¹ to about 1.2 mg L⁻¹, and to prevent convection and limit gas diffusion (Wiengweera et al., 1997). The water level in the unflooded tray was kept at 2 to 3 cm deep. To understand the hypoxic conditions, the dissolved oxygen level was measured with a DO meter (CM-51, HORIBA Ltd., Japan). All seedlings were grown in a growth chamber (220 μmol m⁻² s⁻¹, 14 hr light/10 hr dark, 23°C), and the experiment was replicated 2 times and we used 3 replicates per line for all lines.

4. **Measurements**

   Seedlings were collected 7 d after treatment, and shoots were separated from roots at the cotyledonary node. The roots were scanned with the WinRHIZO software (Regent Instruments Inc.), and total root length (minus the hypocotyl), root surface area, and average root diameter were analyzed. Each fraction was dried to a constant weight at 70°C to determine dry weight.

   The rate of growth inhibition was calculated as:

   $$\frac{[(\text{control value} – \text{flooded value}) / \text{control value}] \times 100\%}{1}$$

5. **Statistical analyses**

   Seeds of each line (100 seeds per line) were measured for 100-seed weight (g) and the data was used for linear correlation among shoot, root, and whole-plant DW of the plants grown in unflooded condition.

   We analyzed shoot DW, root DW, whole-plant DW, root DW / shoot DW, total root length, root surface area, and average root diameter by two-way analysis of variance (ANOVA) to determine the main effects and interactions between lines and treatments.

   The rate of inhibition of root DW was used for linear correlation with the rates of inhibition of total root length, root surface area, and average root diameter.

**Results**

1. **Effects of flooding on dry matter production**

   We examined the response of 92 soybean lines to
flooding, especially root development, by a flooding assay using a capillary watering culture system with a 0.1% agarose solution (Fig. 1). DWs of each plant part and three root traits (total root length, root surface area, and average root diameter) were determined 7 d after flooding. For hypoxic conditions, the dissolved oxygen level in the solution was 1.25 ± 0.084 mg L⁻¹ at the beginning of the flooding treatment, and decreased to 0.72 ± 0.053 mg L⁻¹ after the flooding treatment (by 42.4% on the average).

Most seedlings developed to the V2 stage (Fehr et al., 1971) in both flooded and control conditions. Flooding decreased the root and whole-plant DWs but not shoot DW, and means varied widely with the line in both flooded and control conditions (Tables 1, 2). The DWs in the control were significantly and positively correlated with the 100-seed weight (shoot DW, \( r = 0.568^{***} \); root DW, \( r = 0.662^{***} \); whole-plant DW, \( r = 0.646^{***} \); \( *** \), \( P < 0.0001 \)). The root-to-shoot DW ratio decreased under flooding (Table 2). The shoot DW inhibition was –8.5% (= promotion) on average (range, –43.5% to 12.2%). The root DW inhibition, in contrast, was positive in all lines and averaged 26.1% (0.8% to 46.2%; Table 2). Two-way ANOVA revealed that both line and treatment influenced all dry matter production (Table 3). There was a significant line × treatment interaction in root and whole-plant DW but not in shoot DW. The root-to-shoot DW ratio was significantly influenced by line, treatment, and line × treatment interaction.

**Fig. 1.** The capillary watering system for soybean. Flooding was conducted at the cotyledon stage for 7 d. A, flooded (water up to hypocotyl); B, unflooded (water only at base); C, schematic views.
2. **Strong suppression of root development by flooding**

Because flooding strongly inhibited root growth, we measured root traits under flooded and unflooded conditions to clarify how the flooding treatment altered soybean root development. Total root length and root surface area were severely reduced by flooding, but root diameter tended to be increased (Table 2). The inhibition of total root length and root surface area was positive in most lines, and ranged from –2.7% to 83.1% and from 2.1% to 76.7%, respectively. Two-way ANOVA revealed that line, treatment, and the line × treatment interaction strongly influenced total root length and root surface area. In contrast, average root diameter was strongly influenced by line and treatment and weakly by line × treatment interaction (Table 3).

3. **Inter-line variation of the flooding effect in soybean root development**

To evaluate inter-line variation of the flooding effect on root development, we analyzed the correlations of the rate of inhibition of root DW with that of total root length, root surface area, and average root diameter. Under flooding, the inhibition of root DW was linearly correlated with that of total root length and root surface area (Fig. 2A, B). G406, Iyodaizu, and Mizumoto Park No. 1, with the lowest rates of inhibition, developed long first-order lateral roots and many fine roots under flooding, which was similar to those in the control (Fig. 3), and their average root diameter did not change under flooding (Fig. 2). In contrast, Toyokomachi, Misuzudaizu, and Tachinagaha, with the highest rates of inhibition of total root length and root surface area, had shorter first-order lateral roots and less branching under flooding than in the control (Fig. 4A, B), a lower proportion of fine root length (< 0.5 mm in diameter; Fig. 4C), and a larger average root diameter under flooding (Fig. 2C).

**Discussion**

Short-term flooding hampered early root development in most soybean lines, although the inhibition varied widely among lines (Table 2, Fig. 2A, B). In previous studies, researchers tended to focus on aboveground parts in evaluating the tolerance or sensitivity to flooding. Our results indicate that it is important to evaluate root development as well. To dissect the effects of flooding on root morphology, we analyzed total root length, root surface area, and average root diameter. We found a linear decrease in root DW with decreasing total root length and root surface area (Fig. 2A, B). This result indicates that the suppression of root development was due mainly to the

Table 2. Effect of the flooding treatment on dry matter production and root traits of soybean seedlings at the cotyledon stage.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Treatment</th>
<th>Average</th>
<th>SD</th>
<th>Max.</th>
<th>Min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot DW (mg)</td>
<td>Flooding</td>
<td>100.6</td>
<td>34.4</td>
<td>185.7</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>93.4</td>
<td>33.5</td>
<td>181.2</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>Inhibition rate (%)</td>
<td>–8.5</td>
<td>11.1</td>
<td>12.2</td>
<td>–43.5</td>
</tr>
<tr>
<td>Root DW (mg)</td>
<td>Flooding</td>
<td>67.3</td>
<td>19.6</td>
<td>100.3</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>94.5</td>
<td>32.6</td>
<td>156.8</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>Inhibition rate (%)</td>
<td>26.1</td>
<td>10.2</td>
<td>46.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Whole-plant DW (mg)</td>
<td>Flooding</td>
<td>168.0</td>
<td>52.3</td>
<td>262.8</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>187.8</td>
<td>64.7</td>
<td>323.0</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>Inhibition rate (%)</td>
<td>9.0</td>
<td>8.8</td>
<td>28.9</td>
<td>–13.7</td>
</tr>
<tr>
<td>Root DW / Shoot DW ratio</td>
<td>Flooding</td>
<td>0.71</td>
<td>0.11</td>
<td>1.02</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.03</td>
<td>0.14</td>
<td>1.36</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Inhibition rate (%)</td>
<td>30.5</td>
<td>8.5</td>
<td>48.3</td>
<td>8.0</td>
</tr>
<tr>
<td>Total root length (cm)</td>
<td>Flooding</td>
<td>155.5</td>
<td>51.7</td>
<td>368.7</td>
<td>58.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>467.9</td>
<td>156.0</td>
<td>917.3</td>
<td>57.7</td>
</tr>
<tr>
<td></td>
<td>Inhibition rate (%)</td>
<td>63.4</td>
<td>15.3</td>
<td>83.1</td>
<td>–2.7</td>
</tr>
<tr>
<td>Root surface area (cm²)</td>
<td>Flooding</td>
<td>30.4</td>
<td>8.6</td>
<td>52.6</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>77.6</td>
<td>25.6</td>
<td>131.3</td>
<td>7.0</td>
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<tr>
<td></td>
<td>Inhibition rate (%)</td>
<td>57.5</td>
<td>13.6</td>
<td>76.7</td>
<td>2.1</td>
</tr>
<tr>
<td>Root average diameter (mm)</td>
<td>Flooding</td>
<td>0.65</td>
<td>0.10</td>
<td>0.89</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.53</td>
<td>0.06</td>
<td>0.69</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Inhibition rate (%)</td>
<td>–23.3</td>
<td>13.2</td>
<td>4.3</td>
<td>–59.7</td>
</tr>
</tbody>
</table>

Z without cotyledon
decrease of root elongation. In fact, flood-tolerant lines continued root elongation under both flooded and control conditions (Figs. 2, 3). In contrast, flooding tended to increase average root diameter (Table 2, Fig. 2C), but flood-tolerant lines showed a similar root morphology under both flooded and control conditions (Fig. 3). Roots of flood-susceptible lines were short and thick, lacking in fine second- and higher-order lateral roots under flooding, and had a lower proportion of fine roots (< 0.5 mm in diameter) under flooding (Fig. 4). We hypothesize that flood-susceptible lines fail to develop fine roots. To prove whether flooding causes such a structural change in all soybeans we need to observe each root part. Our results show that root DW, root length, and root surface area are valuable indices of flood-tolerance of soybean plants.

The correlations of root DW with the three root traits help us to discriminate the degree of flood-tolerance in each soybean line (Fig. 2). We did not analyze the effect of flooding on shoot DW further since it was not clear. G406, Iyodaizu, and Mizumoto Park No. 1 were flood-tolerant (Fig. 2), and their root development under flooding was similar to that under control conditions (Fig. 3). Roots of flood-susceptible lines were short and thick, lacking in fine second- and higher-order lateral roots under flooding, and had a lower proportion of fine roots (< 0.5 mm in diameter) under flooding (Fig. 4). We hypothesize that flood-susceptible lines fail to develop fine roots. To prove whether flooding causes such a structural change in all soybeans we need to observe each root part. Our results show that root DW, root length, and root surface area are valuable indices of flood-tolerance of soybean plants.

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The flood tolerance of soybean has been estimated from the difference in whole-plant biomass between flooded
The response of shoot growth, in contrast, was ambiguous, and the shoot DW tended to increase by flooding in some lines. Bacanamwo and Purcell (1999a) reported that the shoot biomass of soybean was similarly unaltered during the first 7 d of flooding, but decreased relative to the control by 21 d. Thus, longer flooding might affect shoot growth in our assay system. Several researchers observed aerenchyma and adventitious roots in flooded soybean plants (Bacanamwo and Purcell, 1999b; Lee et al., 2003; Shimamura et al., 2003a, b; Henshaw et al., 2007a). Both of them can help to restore the oxygen supply to the submerged parts (Bacanamwo and Purcell, 1999b; Visser and Voesenek, 2004). In the present experiment, all lines except G406 formed aerenchyma, though no or few
adventitious roots, in the flooded hypocotyl region (data not shown).

Domestication of plant species was achieved mainly through the observation and selection of aboveground organs, not the roots (Waines and Ehdaie, 2007). We revealed genetic variation in the effects of flooding on root development and discriminated the flood-tolerant and flood-susceptible lines. Flood-tolerant lines showed similar root growth in both flooded and control conditions, whereas flood-susceptible lines show severe inhibition of root growth under flooding. This new knowledge will be useful for understanding the effects of flooding in plants and for QTL analysis and identification of genes related to root development under flooding.

Fig. 4. Root development of flood-susceptible soybean lines after 7 d in (A) unflooded (control) and (B) flooded conditions. Bars = 5 cm. (C) Proportion of root length by diameter.

References


Bacanamwo, M. and Purcell, L.C. 1999a. Soybean dry matter and N accumulation responses to flooding stress, N sources, and


* In Japanese with English abstract.